

weight, would allow for a reproducible and controlled performance. There is thus a need for a reliable and reproducible means of gelatin production that provides a homogeneous product with controlled characteristics.

At page 6, replace the paragraph beginning at line 17 with the following paragraph.

In one aspect, the present invention provides a composition comprising recombinant gelatin, wherein the recombinant gelatin comprises a homogeneous mixture of recombinant gelatin polypeptides. In another aspect, the recombinant gelatin comprises a heterogeneous mixture of recombinant gelatin polypeptides.

At page 30, replace the paragraph beginning at line 37 with the following paragraph.

The present invention provides methods for designing recombinant gelatins with the physical properties desired for particular applications. In one embodiment, the present invention provides recombinant gelatins comprising uniform molecules of a specified molecular weight or range of molecular weights, and methods for producing these recombinant gelatins. Such homogeneous and uniform materials are advantageous in that they provide a reliable source of product with predictable performance, minimizing variability in product performance and in manufacturing parameters. Currently, gelatin from different lots must sometimes be blended in order to produce a mixture with the desired physical characteristics, such as the viscosity or gel strength, etc., provided by a particular molecular weight or molecular weight range.

At page 38, replace the paragraph beginning at line 13 with the following paragraph.

In various aspects, the present invention provides recombinant gelatin that is non-hydrolyzed, fully hydrolyzed, or hydrolyzed to varying degrees, such as gelatins that are a mixture of hydrolyzed and non-hydrolyzed products. Additionally, the present invention provides methods of producing recombinant gelatins with varying degrees of hydrolysis. (See Examples 9 and 10.) Gelatin hydrosylates are typically cold water-

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conclude

soluble and are used in a variety of applications, particularly in the pharmaceutical and food industries, in which a gelatin with non-gelling properties is desirable. Gelatin hydrolysates are used in the pharmaceutical industry in film-forming agents, microencapsulation processes, arthritis and joint relief formulas, tableting, and various nutritional formulas. In the cosmetics industry, gelatin hydrolysates are used in shampoos and conditioners, lotions and other formulations, including lipsticks, and in fingernail formulas, etc. Gelatin hydrolysates appear as nutritional supplements in protein and energy drinks and foods; are used as fining agents in wine, beer, and juice clarification; and are used in the microencapsulation of additives such as food flavorings and colors. Gelatin hydrolysates are used in industrial applications for their film-forming characteristics, such as in coatings of elements in semiconductor manufacture, etc.

At page 39, replace the paragraph beginning at line 18 with the following paragraph.

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In one aspect of the present invention, it is desirable to create a homogeneous gelatin composed of fragments synthesized from collagen constructs lacking platelet activation regions. Such gelatin could be included, for example, in products associated with anastomosis and vascular grafting, etc., including coatings for stent and graft devices. Such products can be associated with deleterious side effects, for example, thrombosis, that can develop in association with the use of such products as a result of the platelet-aggregating regions present in the collagenous product. In one aspect, the present invention provides for a method of producing a recombinant gelatin which can provide support for cell attachment when used in a stent or similar device, but which does not include platelet-reactive regions, thus minimizing the risk of platelet aggregation. (See Example 2.) Therefore, the present invention provides in one embodiment for a stent coating comprising recombinant gelatin. In a preferred embodiment, the recombinant gelatin is recombinant human gelatin. In some instances, such as various wound care applications, it could be desirable to provide recombinant gelatin comprising domains capable of inducing specific aggregating activities.

At page 39, replace the paragraph beginning at line 33 with the following paragraph.

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A gelatin of the present invention could be expressed from collagen constructs that did not encode the regions recognized by the $\alpha 2\beta 1$ receptor, or from constructs with one or with multiple copies of such regions, thus providing a homogeneous and consistent gelatin product without or with reduced platelet aggregation and activation. In one aspect, the present invention provides for the production of recombinant gelatin, either through direct expression of gelatin or through processing of gelatin from collagenous polypeptides, through the use of highly efficient recombinant expression. The present production methods, as opposed to current methods of extraction, offer extreme flexibility, as any one of a number of expression systems can be used. The production material is accessible, for example, in yeast or plant biomass. Secretion in certain production systems can be optimized, for example, by dictating the uniform size of particular gelatin molecules to be produced according to the present methods. In various embodiments, the present gelatins or the polypeptides from which these gelatins are derived, are produced in expression systems including, but not limited to, prokaryotic expression systems, such as bacterial expression systems, and eukaryotic expression systems, including yeast, animal, plant, and insect expression systems. Expression systems such as transgenic animals and transgenic plants are contemplated.

At page 40, replace the paragraph beginning at line 15 with the following paragraph.

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~~The present invention provides for expression of at least one polynucleotide encoding a gelatin or a polypeptide from which gelatin can be derived in a cell. In one embodiment, the present invention provides for the expression of more than one polynucleotide encoding a gelatin or a polypeptide from which gelatin can be derived in a cell, such that recombinant gelatin containing homogeneous or heterogeneous polypeptides is produced. The present invention further provides for expression of a polynucleotide encoding a collagen processing or post-translational enzyme or subunit thereof in a cell. Different post-translational modifications, and different post-translational enzymes, e.g., prolyl~~

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~~hydroxylase, lysyl hydroxylase, etc., can effect, for example, Bloom strength and other physical characteristics of the present gelatins.~~

At page 40, replace the paragraph beginning at line 34 with the following paragraph.

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Nucleic acid sequences encoding collagens have been generally described in the art. (See, e.g., Fuller and Boedtker (1981) Biochemistry 20:996-1006; Sandell et al. (1984) J Biol Chem 259:7826-34; Kohno et al. (1984) J Biol Chem 259:13668-13673; French et al. (1985) Gene 39:311-312; Metsaranta et al. (1991) J Biol Chem 266:16862-16869; Metsaranta et al. (1991) Biochim Biophys Acta 1089:241-243; Wood et al. (1987) Gene 61:225-230; Glumoff et al. (1994) Biochim Biophys Acta 1217:41-48; Shirai et al. (1998) Matrix Biology 17:85-88; Tromp et al. (1988) Biochem J 253:919-912; Kuivaniemi et al. (1988) Biochem J 252:633-640; and Ala-Kokko et al. (1989) Biochem J 260:509-516.) See also co-pending, commonly-owned application U.S. Patent Application Serial No. 09/709,700, entitled "Animal Collagens and Gelatins," filed 10 November 2000, incorporated herein by reference in its entirety.)

At page 58, replace the paragraph beginning at line 19 with the following paragraph.

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Gelatin in various edible forms has long been used in the food and beverage industries. Gelatin is used widely in various confectionery and dessert products, particularly in puddings, frostings, cream fillings, and dairy and frozen products. Gelatin serves as an emulsifier and thickener in various whipped toppings, as well as in soups and sauces. Gelatin is used as a flocculating agent in clarifying and fining various beverages, including wines and fruit juices. Gelatin is used in various low and reduced fat products, such as mayonnaise and salad dressings, as a thickener and stabilizer, and appears elsewhere as a fat substitute. Gelatin is also widely used in microencapsulation of flavorings, colors, and vitamins. Gelatin can also be used as a protein supplement in various high energy and nutritional beverages and foods, such as those prevalent in the weight-loss and athletic industries. As a film-former, gelatin is used in coating fruits, meats, deli items, and in various confectionery products, including candies and gum, etc.

At page 58, replace the paragraph beginning at line 32 with the following paragraph.

Q10 In the cosmetics industry, gelatin appears in a variety of hair care and skin care products. Gelatin is used as a thickener and bodying agent in a number of shampoos, mousses, creams, lotions, face masks, lipsticks, manicuring solutions and products, and other cosmetic devices and applications. Gelatin is also used in the cosmetics industry in microencapsulation and packaging of various products.

At page 59, replace the paragraph beginning at line 25 with the following paragraph.

Q11 Gelatin has also been a valuable substance for use in various laboratory applications. For example, gelatin can be used in various cell culture applications, providing a suitable surface for cell attachment and growth, e.g., as a coating for plates, flasks, microbeads, or other substrates, or providing a suitable protein source in growth media. Hydrolyzed or low gel strength gelatin is used as a biological buffer in various processes, for example, in coating and blocking solutions used in assays such as enzyme-linked immunosorbent assays (ELISAs) and other immunoassays. Gelatin is also a component in various gels used for biochemical and electrophoretic analysis, including enzymography gels.

At page 60, replace the paragraph beginning at line 18 with the following paragraph.

Q12 In addition to providing a gelatin material without the immunogenicity and infectivity issues associated with animal-derived materials, the present invention allows for a reproducible source of consistent product. Specifically, the present gelatins can be presented as a homogeneous mixture of identical molecules. The physical characteristics desired in a particular medical application can be specifically introduced and achieved consistently. The present invention is thus able to provide a reliable and consistent product will minimize variability associated with the availability and use of current gelatin products.

At page 61, replace the paragraph beginning at line 24 with the following paragraph.

913 The recombinant gelatin of the present invention can also be used as a stabilizer in various pharmaceutical products, for example, in drugs or vaccines. (See, e.g., co-pending, commonly-owned U.S. Patent Application Serial No. 09/710,249, entitled "Recombinant Gelatins in Vaccines," filed 10 November 2000, incorporated herein by reference in its entirety.) Therefore, in one embodiment, the present invention provides a stabilizing agent comprising recombinant gelatin, wherein the stabilizer is suitable for use in pharmaceutical applications. In a preferred embodiment, the recombinant gelatin is recombinant human gelatin.

At page 69, replace the paragraph beginning at line 13 with the following paragraph.

914 Gelatin is also used in microencapsulation of various flavors, colors, and other additives, and of vitamins.

At page 69, replace the paragraph beginning at line 16 with the following paragraph.

915 Specifically contemplated are various recombinant gelatins that can be used as stabilizing agents, thickening agents, film-forming agents, binding agents, edible coatings, gelling agents, protein supplements, emulsifying agents, microencapsulants for colors, flavors, and vitamins, etc., and can be used in various food supplements, including nutritional and diet supplements, and fat substitutes. In one embodiment, the gelatin of the present invention is used in the processing or packaging of, or as a component in, foods prepared for consumers with Kosher, Halal, vegetarian, or other diets that restrict the ingestion of food containing specific animal-source products.

At page 71, replace the paragraph beginning at line 11 with the following paragraph.

916 The distinctive properties of gelatin, including its ability to serve as a protective colloid, and to alter its electrical charge with changes in pH, combine to make gelatin a material

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suitable for use in microencapsulation. Gelatin and its derivatives can thus be used in a variety of microencapsulation devices and techniques, for example, in the microencapsulation of inks for carbon-free paper; fragrances for advertising and sample manufacture; chemicals used in multi-component adhesives; and vitamins and nutritional supplements. The microencapsulation capabilities of gelatin and its derivatives are also useful in the manufacture of packaging materials, including packaging allowing minimal permeability for oxygen, aromas, and water vapor. Gelatin is thus widely used in flexible packaging, such as packaging for food, pharmaceuticals, and other sensitive products.

At page 72, replace the paragraph beginning at line 34 with the following paragraph.

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Recombinant gelatins consisting of biologically active regions of collagen type III, for example, can be prepared as microfibers that consist of a uniformity, purity, and reproducibility unattainable with current collagen and gelatin sources. Microfibers derived from the present recombinant gelatin can be presented on substrates, e.g., arrays or chips, used to screen for compounds that prevent platelet aggregation through interaction with, e.g., type III collagen, or any other fibril-forming collagen. Chemical compounds, small molecules, peptides, or other biological molecules (such as antibodies) can be screened for their ability to prevent, reduce, or slow the process of clot formation or platelet aggregation, mediated by platelet interactions with specific regions within a collagen fiber, such as, for example, RGD sequences. Additionally, microarrays would also be useful for examination of the interaction of different types of integrins with various regions of collagens and gelatin microfibers. Microfibers produced from recombinant gelatins from any of the fibril-forming collagens, e.g., collagen type I, type II, type III, type V, or type XI, could be used in screening for collagen-induced platelet aggregation antagonists.

At page 74, replace the paragraph beginning at line 28 with the following paragraph.

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The distinctive properties of gelatin, including its ability to serve as a protective colloid, and to alter its electrical charge with changes in pH, combine to make gelatin a material